1: J Mol Biol 1993 Sep 20;233(2):219-30 Related Articles, Books, LinkOut

Information analysis of sequences that bind the replication initiator RepA.

Papp PP, Chattoraj DK, Schneider TD.

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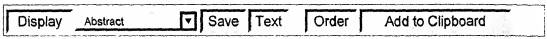
The replication initiator protein RepA of plasmid P1 can bind to 14 sites on the plasmid. These sites are variously used to autoregulate RepA synthesis and for initiation and control of DNA replication. Analysis of information (degree of conservation) at the sites revealed three sequence patches of high conservation. By saturation mutagenesis, the conservation at the outer two patches was found to contribute to RepA binding more critically. The guanine bases that are likely to contact RepA through the major groove were identified by methylation interference and methylation protection experiments. These bases mapped to the outer two patches and were separated by one turn of the helix. Therefore, they belong to major grooves on the same face of DNA. All backbone contacts of the protein, determined by hydroxyl radical footprinting, also mapped to the same face. We conclude from this that RepA binds to its site on one face of the DNA. Information analysis of binding sites for several prokaryotic repressors and activators, where the nature of DNA-protein contacts are known, revealed a correlation between the positions of high conservation and the positions of major grooves that faced the protein. The middle patch of high conservation in the RepA binding sites is an exception since in this region a minor groove is likely to face the protein. The simplest model for minor groove contacts suggests that in B-form DNA a T.A base-pair cannot easily be distinguished from an A.T pair by inspection of the minor groove. Yet in the RepA site, a T-->A mutation in the middle patch significantly affects binding. Therefore, the simplest models for both minor and major groove contacts are unlikely. It is possible that the patch determines the proper conformation of the site and thereby contributes to recognition indirectly.

PMID: 8377199 [PubMed - indexed for MEDLINE]



Excess information at bacteriophage T7 genomic promoters detected by a random cloning technique.

Schneider TD, Stormo GD.

National Cancer Institute, Laboratory of Mathematical Biology, Frederick, MD 21701.

In our previous analysis of the information at binding sites on nucleic acids, we found that most of the sites examined contain the amount of information expected from their frequency in the genome. The sequences at bacteriophage T7 promoters are an exception, because they are far more conserved (35 bits of information content) than should be necessary to distinguish them from the background of the Escherichia coli genome (17 bits). To determine the information actually used by the T7 RNA polymerase, promoters were chemically synthesized with many variations and those that function well in an in vivo assay were sequenced. Our analysis shows that the polymerase uses 18 bits of information, so the sequences at phage genomic promoters have significantly more information than the polymerase needs. The excess may represent the binding site of another protein.

PMID: 2915926 [PubMed - indexed for MEDLINE]

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Redox-dependent Shift of OxyR-DNA Contacts Along an Extended DNA-Binding Site: a Mechanism For Differential Promoter Selection

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The redox-sensitive OxyR protein activates the transcription of antioxidant defense genes in response to oxidative stress and represses its own expression under both oxidizing and reducing conditions. Previous studies showed that OxyR-binding sites are unusually long with limited sequence similarity. Here, we report that oxidized OxyR recognizes a motif comprised of four ATAGnt elements spaced at 10 bp intervals and contacts these elements in four adjacent major grooves on one face of the DNA helix. In contrast, reduced OxyR contacts two pairs of adjacent major grooves separated by one helical turn. The two modes of binding are essential for OxyR to function as both an activator and a repressor in vivo. We propose that specific DNA recognition by an OxyR tetramer is achieved with four contacts of intermediate affinity allowing OxyR to reposition its DNA contacts and target alternate sets of promoters as the cellular redox state is altered.

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- Hausladen, Alfred, Privalle, Christopher T., Keng, Teresa, DeAngelo, Joseph, and Stamler, Jonathan S. (1996). Nitrosative Stress: Activation of the Transcription Factor OxyR. *Cell* 86:719 **[Summary] [Full Text]**

1: Plasmid 1998 Sep;40(2):164-8 Related Articles, Books, Nucleotide, LinkOut

IDEAL

A versatile prokaryotic cloning vector with six dual restriction enzyme sites in the polylinker facilitates efficient subcloning into vectors with unique cloning sites.

Sage DR, Chillemi AC, Fingeroth JD.

Division of Infectious Diseases, Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.

In large and complex vectors a single restriction enzyme recognition site may be available for introduction of additional DNA requiring the development of linker fragments to create compatible insertion sites. This technology can be time consuming and costly. We describe the construction of a simple phagemid, pSFI, with a polylinker that contains six pairs of dual, rare-cutting, restriction enzyme recognition sites (NotI, SpeI, EcoRV, PstI, SacII, EagI) with multiple unique sites between each pair. This has permitted rapid subcloning of DNA with creation of single flanking restriction enzyme sites. pSFI was used to expedite transfer of viral genes to a LacZ-inducible expression vector and to an adenovirus expression cassette for production of replication-defective virus. The use of this phagemid has facilitated complex vector manipulations and is a valuable adjunct to the family of multifunctional cloning vectors.

PMID: 9735318 [PubMed - indexed for MEDLINE]

Preferential cleavage by restriction endonuclease HinfIII.

Piekarowicz A.

The efficiency of endonucleolytic scission by restriction endonuclease HinfIII varies markedly for different recognition sites. The relative frequencies of cleavage at these sites have been determined on the basis of analysis of specific unit length linear molecules formed. The efficiency of restriction reaction depends also on the number of recognition sites in the DNA substrate. Cleavage by HinfIII in the absence or presence of S-adenosylmethionine is observed only when at least three recognition sites are present. HinfIII also shows preferential methylation of certain sites observable even for a substrate with one recognition site. The nucleotide sequences at sites cleaved or methylated at high frequency have been compared.

PMID: 6099947 [PubMed - indexed for MEDLINE]

1: Biotechniques 1993 Aug;15(2):310-5 Related Articles, Books

Protecting recognition sequences on DNA by a cleavage-deficient restriction endonuclease.

Xu SY, Schildkraut I.

New England Biolabs, Beverly, MA 01915.

This report describes the use of a biochemical tool that has been developed to aid in the manipulation of DNA. A DNA binding-proficient and cleavage-deficient BamHI mutant protein, E113K, was used in vitro to protect its recognition sequence (5'-GGATCC-3') against the catalytic action of site-specific endonuclease, exonuclease and methylase. In vitro conditions are reported here in which the E113K protein protects BamHI sites (5'-GGATCC-3') from cleavage by BamHI endonuclease or Sau3AI endonuclease (5'-GATC-3'); protects a neighboring restriction site 5'-CCCGGG-3' from SmaI endonuclease digestion; blocks methylation of 5'-GGATCC-3' by Dam methylase (5'-GATC-3'); and blocks Bal31 exonuclease progression at a BamHI site. The Bal31 procedure could be used to generate unidirectional deletions of a DNA fragment. The use of mutant endonucleases that are binding-proficient and cleavage-deficient to shield DNA from nuclease digestion or methylase modification expands the repertoire of methods to manipulate DNA in vitro.

PMID: 8396948 [PubMed - indexed for MEDLINE]